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MODULATION OF BONE REMODELLING VIA MECHANICALLY ACTIVATED ION CHANNELS.

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Description of Research

It is becoming increasingly evident that mechanical loading plays a critical role in bone homeostasis. Removal of mechanical strain resulting from prolonged periods of weightlessness, immobilization, or skeletal unloading induces a rapid loss of bone leading to a pathologic condition termed disuse osteoporosis. In contrast, physical exercise or exogenous mechanical loading has been shown to maintain bone homeostasis during immobilization or weightlessness and in some cases even increase bone mass. Both *in vivo* and *in vitro* studies have indicated that the osteoblast is the key cell in sensing mechanical strain. Tetracycline labelling of growing rats subjected to weightlessness has indicated that bone formation decreases while osteoclasts number and bone resorption rates stay the same. Similarly *in vitro* studies have demonstrated that osteoblasts respond to mechanical strain by increasing intracellular levels of second messengers as well as the production of markers known for osteogenic activity. While there is a large amount of literature illustrating the positive effects of mechanical stimulation on bone formation, the signaling mechanisms by which physical stimulus is converted into a cellular response is still unclear. We have characterized a mechanosensitive cation nonselective channel in the osteoblast which we hypothesize is the initial signaling mechanism for mechanotransduction. The major thrust of this grant has been the study of the interaction of mechanical stimulus with these channels and the role that these channels play in osteoblastic function.

To obtain the aims of this research we have combined patch clamp technology with modern methods of cell biology to examine the role of these channels in the normal function of the osteoblast *in vitro*. To date we have found that PTH modulates SA-cat channel kinetics via two distinct mechanisms; 1) increasing channel open probability through the actin cytoskeleton and 2) increasing single channel conductance through the typical second messengers of PTH, cAMP and intracellular calcium. During this reporting period we have focused on the effects of mechanical strain on channel characteristics as well as osteoblastic function. Furthermore using an antisense ogliodeoxynucleotide strategy, we have been able to identify the gene responsible for SA-cat channel expression in the UMR-106.01 osteoblast-like osteosarcoma cell.

Accomplishments

1. We have found that osteoblast-like osteosarcoma cells subjected to chronic, intermittent mechanical strain respond to additional strain with a significant increase in whole cell conductance when compared to non-strain control cells. This increase could be blocked by the mechanosensitive channel blocker gadolinium and corresponds to a three to five-fold increase in SA-cat channel activity. Chronic, intermittent strain also increased sensitivity of the channels to stretch and induced spontaneous channel activity.

2. Using the same mechanical strain regimen as used in accomplishment #1, we analyzed the expression and production of bone matrix proteins in the human osteoblast-like

osteosarcoma cell line OHS-4. Type I collagen expression and production were increased within 24 hours of strain application. Significant increases in osteopontin expression and osteocalcin secretion were also observed after three to four days of mechanical strain. Surprisingly, the increases in osteopontin and osteocalcin were independent, and synergistic, to 1,25$(OH)_2$ vitamin D stimulation .

3. As discussed in accomplishment #1, chronically strained osteoblast respond to additional mechanical strain with a large increase in whole cell conductance. Using antisense oligodeoxynucleotides (ODN) derived from nonconserved sequences in the S6 region of the fourth membrane spanning domain of the α_1 subunit of voltage sensitive calcium channels, we found that α_{1c} antisense ODN completely abolished the mechanically induced increase in whole cell conductance. Antisense ODNs to α_{1s} and α_{1d} as well as sense ODN for the α_{1c} subunit had no effect on the strain induced increase in whole cell conductance. Cell attached single channel studies demonstrated that the antisense ODN to the α_{1c} subunit completely blocked the SA-cat single channel response to strain induced by hypotonicity while the α_{1s} antisense ODN did not affect SA-cat channel activity.

Significance of Accomplishments

Mechanical strain plays an important role in bone homeostasis promoting bone formation and remodelling activity, resulting in a net increase in bone mass. Furthermore, *in vitro* studies have also demonstrated increased osteoblastic function with mechanical strain. However, the mechanisms by which osteoblasts and other bone forming cells sense these mechanical perturbations have yet to be identified. We have demonstrated that when osteoblasts are subjected to cyclic, chronic, mechanical strain SA-cat channel kinetics are significantly modulated. This change in channel activity relates to large increases in whole cell conductance when the osteoblast is further stimulated. We have previously shown that this channel is nonselective for cation and is capable of conducting calcium ions into the cell. Jones, et al. (Biomaterials 12;101, 1991) have shown that intracellular calcium increases within milliseconds of the onset of mechanical strain suggesting a mechanosensitive, calcium-conductive channel maybe the primary response element for mechanotransduction. Interestingly, Lanyon and associates have demonstrated that dynamic, but not static mechanical strain produces osteogenic effects in *in vivo* preparations. Our studies have shown that when osteoblasts are strained in the patch configuration without prior conditioning strain, little change is observed in whole cell conductance. These data would suggest that, like *in vivo* observations, multiple stimulation events are required to modulate the SA-cat channel and that chronic, intermittent mechanical strain "primes" the channel to respond to additional strain and promote osteogenic activity.

To determine if the strain regimen used in the study discussed in accomplishments #1 was sufficient to induce an osteogenic response, bone matrix protein expression and production were evaluated. Chronic, cyclic mechanical strain increased expression and/or production of all of the matrix proteins we examined. These observations strengthened, but did not confirm, our hypothesis that the SA-cat channel is an integral part of the signaling mechanisms for the osteogenic response to mechanical strain. One interesting observation made during these studies was that mechanical strain increased osteopontin and osteocalcin expression and production independent of 1,25 dihydroxyvitamin D stimulation. Previously these matrix proteins were thought to be only increased with vitamin D stimulation. However, when both vitamin D and mechanical strain were applied a synergistic response was observed. These data suggest that mechanical strain alters osteoblastic function through a separate mechanism from vitamin D stimulation.

Using homology based reverse transcriptase prolimerase chain reaction, Barry and Friedman have isolated partial cDNA clones to three α_1 subunits of calcium channels in UMR-106.01 osteoblast-like osteosarcoma cells. They found unique conservation of alternative splicing across each of the α_1 subunit genes in this cell line. Previously, they have found that antisense ODN's to the α_{1c} subunit blocked the volume regulatory response to hypotonic swelling in renal distal tubule cells. To determine if a similar inhibition would occur in the UMR-106.01 cell, we employed an antisense strategy to demonstrate that the α_{1c} subunit was responsible for the whole cell conductance increase associated with chronic strain. We found that the antisense ODN's to the α_{1c} subunit but not the other two subunits of calcium channels did indeed block the whole cell conductance response. Furthermore, the antisense ODN's to the α_{1c} subunit also blocked single channel activity of the SA-cat channel. These studies indicate that the SA-cat channel maybe an alternatively spliced molecule, similar to the voltage-operated calcium channels found in other tissues. The significance of this observation is exciting. Using molecular techniques, we can now examine the levels of channel protein as well as mRNA in cells which have been mechanical strained as well as cell which experience weightlessness or unloading. We are currently cloning the channel protein as well as introducing a promoter region onto the antisense ODN's to knock out this channel over a long period of time and therefore examine the results in osteogenic activity in both control and transfected osteoblasts.

Publications

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